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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12N 5/00, 15/00, A61K 49/00, G01N 33/00 — A1

(11) International Publication Number: WO 95/03397

(43) International Publication Date: 2 February 1995 (02.02.95)

(21) International Application Number: PCT/US94/08110

(22) International Filing Date: 19 July 1994 (19.07.94)

(30) Priority Data:
096,944 22 July 1993 (22.07.93) US

(60) Parent Application or Grant
(63) Related by Continuation
US 096,944 (CON)
Filed on 22 July 1993 (22.07.93)

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(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: TRANSGENIC ANIMAL MODEL FOR COGNITIVE DISORDERS

(57) Abstract

Transgenic mice with a human interleukin 1β gene is provided. The transgenic mice may be used to evaluate compounds affecting Alzheimer's Disease and other cognitive disorders.

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TITLE OF THE INVENTION

TRANSGENIC ANIMAL MODEL FOR COGNITIVE DISORDERS

FIELD OF THE INVENTION

The present invention relates to the expression of human interleukin-1β in brain tissues of transgenic mice.

BACKGROUND OF THE INVENTION

This is a continuation of U.S. Serial No. 08/096,944 filed July 22, 1993, now pending.

Alzheimer's Disease (AD) is a neurological disorder affecting the population over 65 years of age. Incidence of the disease increases from less than 1% at age 60-65, to 5% at age 75, to as high as 47% at age 85. As a result 60% to 80% of all cases of dementia in persons over age 65 are caused by AD. Afflicted individuals exhibit impaired cognitive function and memory. AD pursues a rapid course leading to mental and physical incapacity before death occurs, generally five to ten years after onset of the symptoms. Currently there is no effective treatment for AD and the biochemical basis of the disease remains obscure.

AD presents itself in late life without clear markers that explain the onset of disease. Viral, environmental and immunological factors have been implicated in the development of AD. In contrast to the late-life onset of AD (sporadic disease), familial AD (FAD) is characterized by a more rapid progression of the disease and an early onset in life (as early as 35 years of age).

The dementia associated with AD correlates to the remarkable atrophy of AD brain, which is widespread throughout the brain, involving the neocortex, hippocampus, parahippocampal structures including the entorhinal cortex, olfactory cortex and bulb, the cholinergic forebrain basal nucleus, the dorsal serotonergic nuclei and the locus ceruleus noradregergic nuclei.

A genetic analysis to identify the gene(s) that cause AD has given insights into the defect, implicating the Amyloid Precursor

Protein (APP) gene. In addition, indirect biochemical evidence hints at the involvement of the APP, which is synthesized in neurons. In the brains of AD patients abnormal structures can be identified associated with degenerating neurons. These structures which contain a proteolytic cleavage product of APP, the βA4 peptide, are referred to as neuritic plaques (NP) and are located within the neocortex and the hippocampus.

NP contain several proteins. The most abundant component is the ~42 amino acid βA4 peptide which is proteolytically cleaved from the APP. The presence of the βA4 is significant since several reports have outlined a potential neurotoxic effect of this peptide in vitro. APP is a transmembrane protein expressed in several tissues. Different spliced forms of the APP mRNA give rise to several proteins. The major forms of APP are known as APP 751, APP 770 and APP 695. APP 751 and 770 encode a 56 amino acid insert known as the kunitz protease inhibitor domain while APP 695 lacks the kunitz domain. APP processing has been extensively studied. The APP can be cleaved at amino acid position 669 (secretase cleavage site at amino acid 16 of the βA4, thus preventing βA4 production) and produces a large secreted peptide known as nexin II (encoding the kunitz protease inhibitor domain, which can inhibit secretase cleavage). Cleavage at amino acids 653 and 695 can lead to release of the βA4 peptide. This cleavage may occur in the endolysosomal compartment. The mutations in the APP outlined above are believed to affect the efficiency of APP processing thus increasing the susceptibility to abnormal degradation of the APP protein.

Interleukin-1 (IL-1) represents a family of three cytokines, IL-1α, IL-1β and IL-1ra. IL-1α and IL-1β are produced principally by macrophages and are commonly acknowledged as major drivers of chronic and acute inflammation. Although IL-1α and IL-1β are only distantly related gene products, they share a complete spectrum of properties (size, 3-D shape, biological activity, cell source). IL-1 receptor antagonist (IL-1ra) is an IL-like molecule which binds and blocks the type I IL-1 receptor, thus inhibiting IL-1α and IL-1β action.

Several lines of evidence implicate IL-1 in AD and in other neurological disorders. First, macrophage infiltrates are found around degenerating neurons in AD. In experimentally induced neuronal degeneration, macrophages are the first cells recruited to the area of cell degeneration. Second, in experimental models of neuronal degeneration by excitatory amino acid administration or focal permanent cerebral ischaemia, administration of IL1-ra reduces degeneration. Third, mutant mice which display gait disorders and other neuropathic traits have macrophages which overproduce IL-1 upon stimulation. Fourth, IL-1 mRNA and activity are found in AD brain tissue. Fifth, APP synthesis is inducible by cytokines, specifically by IL-6. Finally, head injury and subsequent inflammation can lead to the early development of AD. Given the abundance of APP production in AD it has been proposed that an IL-1/IL-6 mediated acute phase response may be responsible for amyloidogenesis during AD.

Experimental studies of the role of human interleukin-1β (hIL-1β)in Alzheimer's disease would be facilitated by the existence of an appropriate animal model. Accordingly, it is an object of the present invention to provide a mouse model in which human interleukin-1β (IL-1β) expression can be increased so as to stimulate the formation of amyloid protein precursor (APP). It is a further object of this invention to provide a mouse model wherein a brain-specific promoter is linked to the hIL-1β gene so as to allow expression of the IL-1β gene solely within the brain. The transgenic mice of the present invention are useful in the identification of compounds that affect APP production and compounds that affect IL-1β mediated neuronal dysfunction.

SUMMARY OF THE INVENTION

A transgenic mouse expressing human interleukin-1β is provided. The transgenic mouse of the invention may be used in the study of Alzheimer's Disease and disorders involving the central nervous system.

DETAILED DESCRIPTION OF THE INVENTION

The term "animal" is used herein to include all vertebrate animals, except humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages. A "transgenic animal" is any animal containing one or more cells bearing genetic information received, directly or indirectly, by deliberate genetic manipulation at a subcellular level, such as by microinjection or infection with recombinant virus. This introduced DNA molecule may be integrated within a chromosome, or it may be extra-chromosomally replicating DNA. The term "germ cell-line transgenic animal" refers to a transgenic animal in which the genetic information was introduced into a germ line cell, thereby conferring the ability to transfer the information to offspring. If such offspring in fact possess some or all of that information then they, too, are transgenic animals.

The information may be foreign to the species of animal to which the recipient belongs, foreign only to the particular individual recipient, or genetic information already possessed by the recipient. In the last case, the introduced gene may be differently expressed than the native gene.

The genes may be obtained by isolating them from genomic sources, by preparation of cDNAs from isolated mRNA templates, by directed synthesis, or by some combination thereof.

To be expressed, the structural gene must be coupled to a promoter in a functional manner. Promoter/regulatory sequences may be used to increase, decrease, regulate or designate to certain tissues or to certain stages of development the expression of a gene. The promoter need not be a naturally occurring promoter. In the preferred embodiment of the invention, the human Thy-1 (hThy-1) promoter is used. The hThy-1 promoter preferentially allows expression of the gene of interest, here the human interleukin-1β predominantly within the brain (Gordon, J., et al., Cell 50:445-452, (1987)).

The "transgenic non-human animals" of the invention are produced by introducing "transgenes" into the germline of the non-human animal. The methods enabling the introduction of DNA into

cells are generally available and well-known in the art; however, the generation of a particular type of transgenic animal requires experimentation. Embryonal target cells at various stages of development can be used to introduce transgenes. Different methods of introducing transgenes are used depending on the stage of development of the embryonal target cell. Generally, the zygote is the best target for microinjection. In the mouse, the male pronucleus reaches the size of approximately 20 μm in diameter which allows reproducible injection of 1-2 pL of DNA solution. The use of zygotes as a target for gene transfer has a major advantage in that in most cases the injected DNA will be incorporated into the host gene before the first cleavage (Brinster, et al., (1985) Proc. Natl. Acad. Sci. USA 82, 4438-4442). Consequently, nearly all cells of the transgenic non-human animal will carry the incorporated transgene. Generally, this will also result in the efficient transmission of the transgene to offspring of the founder since 50% of the germ cells will harbor the transgene. Microinjection of zygotes is the preferred method for incorporating transgenes in practicing the invention.

Retroviral infection can also be used to introduce a transgene into non-human animal. The developing non-human embryo can be cultured in vitro to the blastocyst stage. During this time, the blastomeres can be targets for retroviral infection (Jaenich, R. (1976) Proc. Natl. Acad. Sci. USA 73, 1260-1264). Efficient infection of the blastomeres is obtained by enzymatic treatment to remove the zona pellucida (Hogan et al., (1986) in Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The viral vector system used to introduce the transgene is typically a replication-defective retrovirus carrying the transgene (Jahner et al., (1985) Proc. Natl. Acad. Sci. USA 82, 6927-6931; Van der Putten et al., (1985) Proc. Natl. Acad. Sci. USA 82, 6148-6152). Transfection is easily and efficiently obtained by culturing the blastomeres on a monolayer of virus-producing cells (Van der Putten, supra; Stewart et al., (1987) EMBO J. 6: 383-388). Alternatively, infection can be performed at a later stage. Virus or virus-producing cells can be

injected into the blastocoele (Jahner et al., (1982) Nature 298: 623-628). Most of the founder animals will be mosaic for the transgene since incorporation occurs only in a subset of the cells which formed the transgenic non-human animal. Further, the founder animal may contain retroviral insertions of the transgene at a variety of positions in the genome; these generally segregate in the offspring. In addition, it is also possible to introduce transgenes into the germ line, albeit with low efficiency, by intrauterine retroviral infection of the midgestation embryo (Jahner et al., (1982) supra).

A third type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells are obtained from pre-implantation embryos cultured in vitro (Evans, M. J., et al., (1981) Nature 292, 154-156; Bradley, A., et al., (1984) Nature 309, 255-258; Gossler, et al., (1986) Proc. Natl. Acad. Sci. USA 83, 9065-9069; and Robertson, et al., (1986) Nature 322, 445-448). Transgenes can be efficiently introduced into ES cells by DNA transfection or by retrovirus-mediated transduction. The resulting transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The ES cells colonize the embryo and contribute to the germ line of the resulting chimeric animal (For review see Jaenisch, R. (1988) Science 240, 1468-1474).

The methods for evaluating the presence of the introduced DNA as well as its expression are readily available and well-known in the art. Such methods include, but are not limited to DNA hybridization to detect the exogenous DNA polymerase chain reaction (PCR), polyacrylamide gel electrophoresis (PAGE) and Western Blots to detect IL-1β protein and the appearance of pathology associated with Alzheimer's Disease.

As used herein, a "transgene" is a DNA sequence introduced into the germline of a non-human animal by way of human intervention such as by way of the methods described below. The transgenes of the invention include DNA sequences which are capable of suppressing cognate endogenous alleles.

Attempts to express human interleukin 1β in transgenic animals have met with minimal success. There may be several reasons for this. First, IL-1β is toxic at relatively low concentrations. Second, IL-1β has been implicated in embryonic development and parturition. Third, IL-1β is produced as a precursor which is processed for release from specific types of cells (e.g., macrophages) by a specific mechanism.

To circumvent the physiological problems associated with overexpression of IL-1β a targetted expression system was developed. The gene encoding human interleukin-1β was placed downstream of the human Thy-1 promoter. The hThy-1 promoter specifically targets mouse brain tissue. Transgenic mice carrying the artificial Thyl-IL-1β gene express hIL-1β in brain tissue. The overexpression of IL-1β may increase the synthesis and processing of amyloid precursor protein.

A potential therapeutic compound for Alzheimer's Disease may be detected by measuring its capacity to block the production of amyloid protein precursor (APP) in these transgenic mice. Such compounds will be formulated in accord with known methods to produce pharmaceutically acceptable compositions. Such compositions may be administered to patients in a variety of standard ways.

The following is presented by way of examples and is not to be construed as a limitation on the scope of the invention.

EXAMPLE 1

Construction of plasmid p12849-57-2

pBSHT1 contains a 8kb EcoRI fragment of human Thy-1 gene (hThy-1) in pBSV (Van Rijs et al., Proc. Natl. Acad. Sci. USA 82:5832-5835, 1985).

The 3.7 kb EcoRI -Bg1II fragment of pBSHT1 containing the hThy-1 promoter and the ATG translation initiation codon was cloned into the EcoRI-BamHI site of plasmid pTZ18u and, the resulting plasmid was called pHZ020.

The 1.6 kb BamHI-Bg1II fragment of pBSHT1, containing the ATG initiation codon, was cloned into the BamHI site of pTZ18u (pHZ021a). A PCR amplication was carried out using pHZ021a as a template and oligos T7 (in pTZ18u backbone) and oHZ002 (at the ATG initiation codon) as primers to generate a 1.3 kb product. The sequence of the T7 primer is 5'-TAA TAC GAC TCA CTA TAG GG-3' (SEQ ID NO: 1).

The sequence of oHZ002 is:
5'-ACG TCG ACT CTA GAA GAT CTT CGA CTC GAG ATC GAT GGT ACC CGG GCA GGT TCA AGC TTC TGG GAT CTC AGT C-3' (SEQ ID NO: 2).

The oHZ002 nucleotides destroyed the ATG codon and introduced a polylinker cloning site in the PCR product, as follows:
5'-ACGTCGACTCTAGAAGATCTTCGACTCGAGATCGATGGT ACCCGGGCAGGTTCAAGCTTCTGGGATCTCAGTC-3'
5'-TCATGGTTCTGGGATCTCAGTC-3' Wild-type

A NcoI partial digestion was performed on pHZ020 for cleavage at the downstream site only. This was followed by a XbaI complete digestion which released the NcoI-XbaI fragment containing the ATG codon. The 1.3 kb PCR product was digested with NcoI-XbaI and inserted into the NcoI-XbaI digested pHZ020 (pHZ022).

A Bg1II linker d(CAGATCTG) was inserted at the SmaI site upstream of the SV40 small T intron of pSV2neo (pHZ023). (Southern, P.J. & Berg, P.J. Mol. Appl. Genet. 1:327, 1982.).

The 1.0 kb SV40 small T intron and polyA was isolated by Bg1II and BamHI digestion of pHZ023 and ligated into the Bg1II digested pHZ022. The resulting plasmid, pHZ024 contains two regulatory elements: the human Thy-1 promoter for brain-specific expression and the SV40 small T intron and polyA sequence for proper expression of the transgene.

In addition, the ATG initiation codon of the hThy-1 gene was mutated and a polylinker cloning site was introduced downstream of the hThy-1 promoter; this permitted insertion of genes to be expressed in transgenic mice.

Plasmid pHZ024 was digested with Cla I and Bg1II which cut within the polylinker located between the hThy-1 promoter and the SV40 small T and poly A sequences. The 6.3- kb Cla I- Bg1II vector fragment was gel-isolated. Two synthetic oligodeoxynucleotides were annealed to form a 86-bp linker with the following structure:

5'-CGATGGAACCATGGAAATCTGCAGGGGACCTTACAG
TCACCTAATCTCTCTCCTTCT
3'- TACCTTGGTACCTTTAGACGTCCCCTGGAATGTCAG
TG GATTAGAGAGAGGAAGA

CATCCTTCTGTTTCATTCAGAGGCAGCCTGC- 3'
GTAGGAAGACAAAGTAAGTCTCCGTCGGACG- 5'
(SEQ ID NO:3)

The 86-base pair (bp) linker contains a ClaI sticky end, 5-bps of untranslated leader sequence from the hThy-1 gene (Seki, et al., PNAS 82, 6657-6661, 1985), ATG codon (underlined), 17-bp of rat IL-1 receptor antagonist (IL-1ra) signal peptide sequence and the remaining 58-bp of the IL-1ra signal peptide sequence from mouse. The chimeric rat/mouse IL-1ra signal peptide sequence was used because the first 17-bp of the mouse sequence was unknown. The gene encoding the mature form of the hIL-1β was constructed using PCR. The template DNA for PCR was plasmid pGEM-Blue/human IL-1β (gift of Andrew Howard, Merck Research Laboratories, Rahway, NJ). This plasmid contains the cDNA encoding the mature form of hIL-1β. pGEM-Blue/human IL-1β was constructed by subcloning the EcoRI-AccI IL-1β DNA fragment from pKK223-3/hIL-1β (Tocci, M.J., et al., Journal of Immunology 138, 1109-1114, 1987) into the EcoRI-AccI digested pGEM-Blue vector (Promega, Inc.). It is apparent to those

skilled in the art that pKK223-3/hIL-1β could also be used as template DNA for PCR. PCR-primers had the following sequences: 5'-GCACCTGTACGATCACTGAACTGC-3' (SEQ ID NO: 4) and 5'-GAAGATCTAGGAAGACACAAATTGCATGGTGAAG-3' (SEQ ID NO: 5).

Following PCR, the 0.5-kb hIL-1β gene fragment was made flush-ended with T4 DNA polymerase, digested with BglII (which cuts after the stop codon), gel-isolated and phosphorylated with T4 polynucleotide kinase.

The 6.3-kb ClaI-BglII pHZ024 vector fragment was ligated with the 86-bp IL-1ra ClaI- blunt linker and the 0.5-kb blunt- BglII mature hIL-1β gene in a three-way ligation. The resulting plasmid p12849-57-2 was sequenced using dideoxynucleotide sequencing.

To prepare the DNA cassette for microinjection, a large-scale CsCl plasmid prep of p12849-57-2 was prepared. The plasmid was digested with EcoRI and XbaI and electrophoresed through a 1% low melting point agarose gel containing 10 ng/ml ethidium bromide. The DNA was visualized using minimal exposure to short-wave UV light and the 5.1-kb DNA band was excised, melted at 65-70°C, phenol/chloroform extracted 3X, chloroform extracted 1X and ethanol precipitated in 0.2 M NaCl. Following several 70% ethanol washes, the DNA was resuspended in 10 mM Tris, 0.25 mM EDTA, pH 7.5 and filtered through a pre-rinsed 0.2 μm cellulose acetate filter. The 5.1-kb, linear DNA fragment 12849-112-2 containing the hThy-1 promoter, rat/mouse IL-1ra signal sequence, mature hIL-1β gene and SV40 intron and poly A was subsequently used for microinjection.

A sample of plasmid p12849-57-2 in a host Escherichia coli deposited under the Budapest Treaty in the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MDDD 20852, USA on _____ and has been assigned accession number _____.

All restriction endonucleases and DNA modifying enzymes were from Boehringer Mannheim, Inc. DNA sequencing was performed using either Sequenase (U.S. Biochemical, Inc.) or ds DNA

Cycle Sequencing Kit (BRL, Inc.). Oligodeoxynucleotides were synthesized on ABI DNA Synthesizer model #381A. PCR was according to Perkin-Elmer, Corp.

EXAMPLE 2

Production of transgenic mice containing human IL-1β under regulation of human Thy-1 promoter

The p12849-57-2 DNA construct of Example 1 containing the human IL-1β driven by the human Thy-1 (hThy-1) promoter was microinjected into the pronucleus of one-cell fertilized mouse embryos obtained from superovulated B6SJL females. The optimal concentration of the DNA used for microinjection was the LD_{50} value derived empirically from toxicity test experiments using several dilutions of the gene construct microinjected into mouse embryos and was 7.5×10^{-9} µg. The embryos injected with 7.5×10^{-9} µg DNA were then surgically reimplanted into the oviducts of pseudopregnant recipient mice and allowed to develop to term. At three to four weeks postnatal, tail samples were taken by clipping off approximately 1 cm of tail for DNA dot blot assay to determine the presence of the transgene. Young pups were closely observed daily starting from PN1 (postnatal day 1) for pathological symptoms. Necropsies and/or biopsies were performed to collect tissue specimens for histological and for expression studies.

EXAMPLE 3

Analysis of transgenic mice

DNA Analysis

The pups of Example 2 derived from microinjected eggs are weaned at about 4 weeks of age. At that time a small segment (about 1 cm long) was removed from the distal end of the tail and used for DNA analysis. Genomic DNA was extracted from the tail samples and applied to Gene Screen Plus® membrane filter using a dot blot

apparatus. The filter was then hybridized with a ^{32}P-labeled probe containing SV40 sequence which is present in the 3' area of the transgene. Since the endogenous mouse DNA does not contain the SV40 sequence, this probe is specific for the transgene and can be used to detect as little as 0.1 copies of the transgene in the mouse genome. Transgenic founders identified by DNA dot blot procedure are bred to produce progeny for further studies.

RNA Analysis

Transgenic animal tissues are analyzed for specific mRNA transcription using RNA-polymerase chain reaction (RNA-PCR). Following dissection, mouse tissues are immediately frozen on dry ice and stored at -70°C. Frozen samples are transferred in liquid nitrogen into a prechilled mortar, pulverized and transferred into tubes on dry ice. Total RNA is extracted as described (RNA Isolation Kit, product #200345; Stratagene, Inc.). Oligodeoxynucleotide primer pairs are synthesized for PCR.

The oligonucleotides are designed to complement DNA sequences located within exons. Each oligonucleotide pair is separated by one or more introns in order to distinguish between genomic DNA and mRNA.

One μg of total RNA (determined by absorbance at 260 nm) is used for each RNA-PCR reaction as described (GeneAmp® RNA PCR Kit, product #N808-0017; Perkin Elmer Cetus, Corp.). The PCR is performed for 35 cycles with the following parameters per cycle: 95°C for 1 min., 48°C for 2 min., 72°C for 2 min.

DNA bands of the appropriate sizes are visualized on a 1.2% agarose gel containing 0.5 μg/ml ethidium bromide. The relative mRNA levels (as determined by RNA-PCR) for the tissues of transgenic animals and a control non-transgenic animal are determined.

Human IL-1β mRNA can also be detected by an RNase protection assay. Brain and other tissues are obtained from transgenic mice for isolation of mRNA. A ^{32}P-labeled antisense RNA is used to hydridize hIL-1β mRNA in a solution hybridization reaction. The

resulting double-stranded molecule is resistant to RNase digestion while unhybridized RNA will be digested by RNase treatment. The protected band can be visualized by autoradiography after separation on a polyacrylamide gel.

For in situ hybridization tissue sections are prepared from frozen brain and hybridized with a labeled oligonucleotide probe specific for hIL-1β mRNA followed by autoradiography.

For protein measurements, blood, plasma or homogenate of brain tissue is used for Enzyme Linked Immunoabsorbant Assay (ELISA) to measure hIL-1β concentrations in the tissues.

For histological studies, transgenic mice are necropsied to obtain brain and other tissues. Tissue samples are typically fixed in 10% formalin in phosphate buffered saline. Fixed tissues are sectioned mounted on glass slides and read.

EXAMPLE 4

Cell culture

The transgenic animals of the invention may be used as a source of cells for cell culture. Brain tissues of transgenic mice are analyzed for the presence of human interleukin-1β, by directly analyzing DNA or RNA or by assaying brain tissue for the protein expressed by the gene. Cells of brain tissues carrying the gene may be cultured using standard culture techniques that are well-known in the art.

EXAMPLE 5

Screening assays

The animals of the present invention may be used to test compounds for the ability to interfere the expression of human interleukin-1β or amyloid precursor protein (APP). An animal is treated with the compound, in parallel with an untreated control animal.

A comparatively lower level of IL-1β or APP in the treated animal is an indication of inhibitory activity of the test compound.

SEQUENCE LISTING

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(ii) TITLE OF INVENTION: TRANSGENIC ANIMAL MODEL FOR COGNITIVE DISORDERS

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TAATACGACT CACTATAGGG 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 73 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ACGTCGACTC TAGAAGATCT TCGACTCGAG ATCGATGGTA CCCGGGCAGG TTCAAGCTTC 60

TGGGATCTCA GTC 73

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 173 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGATGGAACC ATGGAATCTG CAGGGGACCT TACAGTCACC TAATCTCTCT CCTTCTTACC 60

TTGGTACCTT TAGACGTCCC CTGGAATGTC AGTGGATTAG AGAGAGGAAG ACATCCTTCT 120

GTTTCATTCA GAGGCAGCCT GCGTAGGAAG ACAAAGTAAG TCTCCGTCGG ACG 173

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCACCTGTAC GATCACTGAA CTGC 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAAGATCTAG GAAGACACAA ATTGCATGGT GAAG 34

WHAT IS CLAIMED IS:

1. A transgenic non-human animal having cells containing a gene encoding a human interleukin-1β protein.

2. The animal of Claim 1 which is a mammal.

3. The animal of Claim 1 which is a rodent.

4. The animal of Claim 1 which is a mouse.

5. The animal of Claim 1 wherein the gene encoding human interleukin-1β is downstream of a human thymidine-1 promoter.

6. The animal of Claim 5 which is a mouse.

7. The mouse of Claim 6 wherein the transgene is p12849-57-2 (ATCC _____).

8. A cell line containing a human interleukin 1β, the cell line being derived from the animal of Claim 4.

9. A method of determining the ability of a compound to interfere with the expression of human interleukin-1β or amyloid precursor protein expression comprising:

(a) treating the transgenic animal of Claim 1 with the compound; and

(b) measuring the expression of human interleukin-1β or amyloid precursor protein in the treated animal.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08110

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 5/00, 15/00; A61K 49/00; G01N 33/00
US CL :435/240.1; 424/9; 800/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.1; 424/9; 800/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Proceedings of the National Academy of Sciences USA, Volume 89, issued June 1992, K. Turksen et al, "Interleukin 6: Insights to its Function in Skin by Overexpression in Transgenic Mice", pages 5068-5072, see entire document.	1-9
Y	Cell, Volume 51, issued 09 October 1987, S. Chen et al, "A Lymphoproliferative Abnormality Associated with Inappropriate Expression of the Thy-1 Antigen in Transgenic Mice", pages 7-19, especially page 9, col. 2, parag. 2 to page 8, col. 1, line 4.	1-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 26 OCTOBER 1994	Date of mailing of the international search report NOV 07 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Crouch, PH.D. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/08110

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Immunology, Volume 138, issued 15 February 1987, M.J. Tocci et al , "Expression in *Escherichia Coli* of Fully Active Recombinant Human Il 1β: Comparison with Native Human IL 1β, pages 1109-1114, especially page 1110, figure 1 and page 1112, col. 2, parag. 1, lines 1-7.	1-9